

SEQUENCE LISTING



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<110> Eck, Jürgen
Schmidt, Arno
Zinke, Holger

<120> Recombinant Fusion Proteins Based on
Ribosome-Inactivating Proteins of the mistletoe Viscum
album

<130> 09282-5

<140> 09/347,064

<141> 1999-07-02

<150> PCT/EP98/00009

<151> 1998-01-02

<150> EP 97 10 0012.0

<151> 1997-01-02

<160> 38

<170> PatentIn Ver. 2.1

<210> 1

<211> 762

<212> DNA

<213> Viscum album

<400> 1
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ctcttgcgtc agtctacgat ccccgctctc gatcgcaaa gatttgtctt ggtggagctc 180
accaaccagg ggggagactc gatcacggcc gccatcgacg ttaccaatct gtacgtcgtg 240
gcttaccagg caggcgacca atcctacttt ttgcgcgacg caccacgcgg cgcggaaacg 300
catctcttca ccggcaccac ccgatcctct ctccattca acggaagcta cctgatctg 360
gagcgatagc ccggacatag ggaccagatc cctctcggtt tagaccaact cattcaatcc 420
gtcacggcgc ttctgtttcc gggcgccagc acgcgtaccc aagctcgttc gattttaate 480
ctcattcaga tgcattccga ggccgccaga ttcaatccca tcttatggag ggctgcgcaa 540
tacattaaca gtggggcgctc atttctgcc aacgtgtaca tgctggagct ggagacgagt 600
tggggccaac aatccacgca agtccagcat tcaaccgatg gcgtttttaa taacccaatt 660
cgggtggcta tacccccggg taacttcgtg acgttgacca atgttcgcga cgtgatcgcc 720
agcttggcga tcatgttgtt tgcattgcga gacgcgccga gt 762

<210> 2

<211> 252

<212> PRT

<213> Viscum album

<400> 2

Met Tyr Glu Arg Ile Arg Leu Arg Val Thr His Gln Thr Thr Gly Glu
1 5 10 15

Glu Tyr Phe Arg Phe Ile Thr Leu Leu Arg Asp Tyr Val Ser Ser Gly
20 25 30

INS
DI
C8

Ser Phe Ser Asn Glu Ile Pro Leu Leu Arg Gln Ser Thr Ile Pro Val
 35 40 45
 Ser Asp Ala Gln Arg Phe Val Leu Val Glu Leu Thr Asn Gln Gly Gly
 50 55 60
 Asp Ser Ile Thr Ala Ala Ile Asp Val Thr Asn Leu Tyr Val Val Ala
 65 70 75 80
 Tyr Gln Ala Gly Asp Gln Ser Tyr Phe Leu Arg Asp Ala Pro Arg Gly
 85 90 95
 Ala Glu Thr His Leu Phe Thr Gly Thr Thr Arg Ser Ser Leu Pro Phe
 100 105 110
 Asn Gly Ser Tyr Pro Asp Leu Glu Arg Tyr Ala Gly His Arg Asp Gln
 115 120 125
 Ile Pro Leu Gly Ile Asp Gln Leu Ile Gln Ser Val Thr Ala Leu Arg
 130 135 140
 Phe Pro Gly Gly Ser Thr Arg Thr Gln Ala Arg Ser Ile Leu Ile Leu
 145 150 155 160
 Ile Gln Met Ile Ser Glu Ala Ala Arg Phe Asn Pro Ile Leu Trp Arg
 165 170 175
 Ala Arg Gln Tyr Ile Asn Ser Gly Ala Ser Phe Leu Pro Asp Val Tyr
 180 185 190
 Met Leu Glu Leu Glu Thr Ser Trp Gly Gln Gln Ser Thr Gln Val Gln
 195 200 205
 His Ser Thr Asp Gly Val Phe Asn Asn Pro Ile Arg Leu Ala Ile Pro
 210 215 220
 Pro Gly Asn Phe Val Thr Leu Thr Asn Val Arg Asp Val Ile Ala Ser
 225 230 235 240
 Leu Ala Ile Met Leu Phe Val Cys Gly Glu Arg Pro
 245 250

<210> 3
 <211> 828
 <212> DNA
 <213> Viscum album

<400> 3
 aggcctgtga tagccgatga tgttacatgt agtgcttcgg aaacctacggt gcggattgtg 60
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 cagttgtggc cctccaagtc caacaatgat ccgaatcagt tgtggacgat caaaagggat 180
 ggaaccattc gatccaatgg cagctgcttg accacgtatg gctatactgc tggcgtctat 240
 gtgatgatct tcgactgtaa tactgctgtg cgggaggcca ctcttggca gatatggggc 300
 aatgggacca tcatcaatcc aagatccaat ctggttttgg cagcatatc tggatcaaaa 360
 ggcactacgc ttacggtgca aacactggat tacacgttgg gacagggtg gcttgccggt 420

aatgataccg cccacgcga ggtgaccata tatgggttca gggacctttg catggaatca 480
 aatggaggga gtgtgtgggt ggagacgtgc gtgagtagcc aaaagaacca aagatgggct 540
 ttgacgggg atggttctat acgcccacaa caaaaccaag accaatgcct cacctgtggg 600
 agagactccg tttcaacagt aatcaatata gttagctgca gcgctggatc gtctgggag 660
 cgatgggtgt ttaccaatga aggggccatt ttgaatttaa agaattgggtt ggccatggat 720
 gtggcgcaag caaatccaaa gctccgccga ataactatc atcctgccac agggaaacca 780
 aatcaaatgt ggcttccgt gccagggtga tatcactagt aaggatcc 828

<210> 4
 <211> 267
 <212> PRT
 <213> Viscum album

<400> 4

Asp Asp Val Thr Cys Ser Ala Ser Glu Pro Thr Val Arg Ile Val Gly
 1 5 10 15

Arg Asn Gly Met Cys Val Asp Val Arg Asp Asp Asp Phe Arg Asp Gly
 20 25 30

Asn Gln Ile Gln Leu Trp Pro Ser Lys Ser Asn Asn Asp Pro Asn Gln
 35 40 45

Leu Trp Thr Ile Lys Arg Asp Gly Thr Ile Arg Ser Asn Gly Ser Cys
 50 55 60

Leu Thr Thr Tyr Gly Tyr Thr Ala Gly Val Tyr Val Met Ile Phe Asp
 65 70 75 80

Cys Asn Thr Ala Val Arg Glu Ala Thr Leu Trp Gln Ile Trp Gly Asn
 85 90 95

Gly Thr Ile Ile Asn Pro Arg Ser Asn Leu Val Leu Ala Ala Ser Ser
 100 105 110

Gly Ile Lys Gly Thr Thr Leu Thr Val Gln Thr Leu Asp Tyr Thr Leu
 115 120 125

Gly Gln Gly Trp Leu Ala Gly Asn Asp Thr Ala Pro Arg Glu Val Thr
 130 135 140

Ile Tyr Gly Phe Arg Asp Leu Cys Met Glu Ser Asn Gly Gly Ser Val
 145 150 155 160

Trp Val Glu Thr Cys Val Ser Ser Gln Lys Asn Gln Arg Trp Ala Leu
 165 170 175

Tyr Gly Asp Gly Ser Ile Arg Pro Lys Gln Asn Gln Asp Gln Cys Leu
 180 185 190

Thr Cys Gly Arg Asp Ser Val Ser Thr Val Ile Asn Ile Val Ser Cys
 195 200 205

Ser Ala Gly Ser Ser Gly Gln Arg Trp Val Phe Thr Asn Glu Gly Ala
 210 215 220

CB
 MS
 DL

Ile Leu Asn Leu Lys Asn Gly Leu Ala Met Asp Val Ala Gln Ala Asn
225 230 235 240

Pro Lys Leu Arg Arg Ile Ile Ile Tyr Pro Ala Thr Gly Lys Pro Asn
245 250 255

Gln Met Trp Leu Pro Val Pro Gly Gly Tyr His
260 265

<210> 5
<211> 48
<212> DNA
<213> Viscum album

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gatgttacat gt 72

<210> 6
<211> 16
<212> PRT
<213> Viscum album

<400> 6
Ser Ser Ser Glu Val Arg Tyr Trp Pro Leu Val Ile Arg Pro Val Ile
1 5 10 15
Ala

<210> 7
<211> 756
<212> DNA
<213> Viscum album

<400> 7
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atcacgcttc tccgagatta tgtctcaagc ggaagctttt ccaatgagat accactcttg 120
cgtcagtcta cgtatcccggt ctccgatgcg caaagatttg tcttgggtga gctcaccaac 180
caggggggag actcgatcac ggccgcccac gacgttaccac atctgtactgt cgtgggttac 240
caagcaggcg accaatccta ctttttgcgc gacgcaccac gcggcgcgga aacgcatttc 300
ttcacgggca ccaccgcatc ctctctccca ttcaacggaa gctaccctga tctggagcga 360
tacgcccggac atagggacca gatccctctc ggtaagacc aactcattca atccgtcacg 420
gcgcttcgtt ttccgggcgg cagcacgcgt acccaagctc gttcgatttt aatcctcatt 480
cagatgatct ccgagggcgc cagattcaat cccatcttat ggagggtctg ccaatacatt 540
aacagtgggg cgtcattttct gccagacgtg tacatgctgg agctggagac gagttggggc 600
caacaatcca cgcaagtcca gcattcaacc gatggcggtt ttaataaccc aattcggttg 660
gctatacccc ccggttaactt cgtgacgttg accaatgttc gcgacgtgat cgccagcttg 720
gcgatcatgt tgtttgtatg cggagagcgg ccatct 756

<210> 8
<211> 252
<212> PRT
<213> Viscum album

<400> 8

Tyr Glu Arg Ile Arg Leu Arg Val Thr His Gln Thr Thr Gly Glu Glu
 1 5 10 15
 Tyr Phe Arg Phe Ile Thr Leu Leu Arg Asp Tyr Val Ser Ser Gly Ser
 20 25 30
 Phe Ser Asn Glu Ile Pro Leu Leu Arg Gln Ser Thr Ile Pro Val Ser
 35 40 45
 Asp Ala Gln Arg Phe Val Leu Val Glu Leu Thr Asn Gln Gly Gly Asp
 50 55 60
 Ser Ile Thr Ala Ala Ile Asp Val Thr Asn Leu Tyr Val Val Ala Tyr
 65 70 75 80
 Gln Ala Gly Asp Gln Ser Tyr Phe Leu Arg Asp Ala Pro Arg Gly Ala
 85 90 95
 Glu Thr His Leu Phe Thr Gly Thr Thr Arg Ser Ser Leu Pro Phe Asn
 100 105 110
 Gly Ser Tyr Pro Asp Leu Glu Arg Tyr Ala Gly His Arg Asp Gln Ile
 115 120 125
 Pro Leu Gly Ile Asp Gln Leu Ile Gln Ser Val Thr Ala Leu Arg Phe
 130 135 140
 Pro Gly Gly Ser Thr Arg Thr Gln Ala Arg Ser Ile Leu Ile Leu Ile
 145 150 155 160
 Gln Met Ile Ser Glu Ala Ala Arg Phe Asn Pro Ile Leu Trp Arg Ala
 165 170 175
 Arg Gln Tyr Ile Asn Ser Gly Ala Ser Phe Leu Pro Asp Val Tyr Met
 180 185 190
 Leu Glu Leu Glu Thr Ser Trp Gly Gln Gln Ser Thr Gln Val Gln His
 195 200 205
 Ser Thr Asp Gly Val Phe Asn Asn Pro Ile Arg Leu Ala Ile Pro Pro
 210 215 220
 Gly Asn Phe Val Thr Leu Thr Asn Val Arg Asp Val Ile Ala Ser Leu
 225 230 235 240
 Ala Ile Met Leu Phe Val Cys Gly Glu Arg Pro Ser
 245 250

<210> 9
 <211> 789
 <212> DNA
 <213> Viscum album

<400> 9
 gatgatgttta cctgcagtgc ttccggaacct acggtgcgga ttgtgggtcg aaatggcatg 60
 tgcgtggagc tcgcagatga cgatttcgcg gatggaaatc agatacagtt gtggccctcc 120

aagtccaaca atgatccgaa tcagttgttg acgatcaaaa gggatggaac cattcgatcc 180
aatggcagct gcttgaccac gtatggctat actgctggcg tctatgtgat gatcttcgac 240
tgtaataactg ctgtgcggga ggccactctt tggcagatat ggggcaatgg gaccatcatc 300
aatccaagat ccaatctggt ttggcgagca tcatctggaa tcaaaaggcac tacgcttacg 360
gtgcaaacac tggattacac gttgggacag ggctggcttg ccggtaatga taccgccccca 420
cgcgagggtga ccatatatgg gttcaggggac ctttgcatgg aatcaaatgg agggagtggt 480
tgggtggaga cgtgcgtgag tagccaaaag aaccaaaagt gggtcttgta cggggatggt 540
tctataccgc ccaaacaaaa ccaagaccaa tgcctcacct gtgggagaga ctccgtttca 600
acagtaatat atatatgttag ctgcagcgct ggcagcgatg ggcagcgatg ggtgtttacc 660
aatgaagggg ccattttgaa tttaagaagt gggttggcca tggatgtggc gcaagcaaat 720
ccaaagctcc gccgaataat catctatcct gccacaggaa aaccaaatac aatgtgctt 780
cccgtgccca

<210> 10
<211> 263
<212> PRT
<213> Viscum album

<400> 10
Asp Asp Val Thr Cys Ser Ala Ser Glu Pro Thr Val Arg Ile Val Gly
1 5 10 15
Arg Asn Gly Met Cys Val Asp Val Arg Asp Asp Asp Phe Arg Asp Gly
20 25 30
Asn Gln Ile Gln Leu Trp Pro Ser Lys Ser Asn Asn Asp Pro Asn Gln
35 40 45
Leu Trp Thr Ile Lys Arg Asp Gly Thr Ile Arg Ser Asn Gly Ser Cys
50 55 60
Leu Thr Thr Tyr Gly Tyr Thr Ala Gly Val Tyr Val Met Ile Phe Asp
65 70 75 80
Cys Asn Thr Ala Val Arg Glu Ala Thr Leu Trp Gln Ile Trp Gly Asn
85 90 95
Gly Thr Ile Ile Asn Pro Arg Ser Asn Leu Val Leu Ala Ala Ser Ser
100 105 110
Gly Ile Lys Gly Thr Thr Leu Thr Val Gln Thr Leu Asp Tyr Thr Leu
115 120 125
Gly Gln Gly Trp Leu Ala Gly Asn Asp Thr Ala Pro Arg Glu Val Thr
130 135 140
Ile Tyr Gly Phe Arg Asp Leu Cys Met Glu Ser Asn Gly Gly Ser Val
145 150 155 160
Trp Val Glu Thr Cys Val Ser Ser Gln Lys Asn Gln Arg Trp Ala Leu
165 170 175
Tyr Gly Asp Gly Ser Ile Arg Pro Lys Gln Asn Gln Asp Gln Cys Leu
180 185 190
Thr Cys Gly Arg Asp Ser Val Ser Thr Val Ile Asn Ile Val Ser Cys

<400> 14

Arg Thr Glu Ser Thr Phe Lys Asn Thr Glu Ile Ser Phe Lys Leu Gly
1 5 10 15

Gln Glu Phe Glu Glu Thr Thr Ala Asp Asn
20 25

<210> 15

<211> 75

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Fig. 20:
Synthetic linker cassette for providing modularity
at the 3' end of rMLB delta lalpha lbeta

<400> 15

caccggtaaa ccgaaccaga tgtggctgcc ggtaccgtag taacgtcct cgtcgaccta 60
gtaaggatcc ctgca 75

<210> 16

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Fig. 20: amino
acid sequence encoded by portion of SEQ ID NO: 15

<400> 16

Thr Gly Lys Pro Asn Gln Met Trp Leu Pro Val Pro
1 5 10

<210> 17

<211> 82

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Fig. 21:
Synthetic linker cassette for providing modularity
at the 3'end of rMLB Delta lalpha lbeta 2gamma
with affinity module ("His-Tag").

<400> 17

ccggtaaacc gaaccagatg tggctgccgg tacccgggtgg tggatatcat caccaccatc 60
accactagta actcctcgga tc 82

<210> 18

<211> 21

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Amino acid
sequence encoded by a portion of SEQ ID NO: 17

<400> 18

Gly Lys Pro Asn Gln Met Trp Leu Pro Val Pro Gly Gly Gly Tyr His
1 5 10 15

His His His His His
20

<210> 19

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Codon exchange
rMLB D23A

<400> 19

catgtgctgt gccgtccgag atgacg 26

<210> 20

<211> 27

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Fig. 22:
Mutagenic oligonucleotides for inactivating
carbohydrate binding sites in rMLB. - 1alpha2
(W38A). -

<400> 20

cagatacagt tggcgccctc caagtcc 27

<210> 21

<211> 61

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Fig. 22:
Mutagenic oligonucleotides for inactivating
carbohydrate binding sites in rMLB. - 1beta (Y68S,
Y70S, Y75S, F79S). -

<400> 21

gctgcttgac cagctgtggc tctactgctg gcgtctctgt gatgatctcc gactgtaata 60
c 61

<210> 22
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Fig. 22:
Mutagenic oligonucleotides for inactivating
carbohydrate binding sites in rMLB. - 1beta1
(D235A). -

<400> 22
gggttgcca tggctgtggc gcaagc

26

<210> 23
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Fig. 22
Mutagenic oligonucleotides for inactivating
carbohydrate binding sites in rMLB. - 2gamma2
(Y249A). -

<400> 23
cgaataatca tcgctcctgc cacagg

26

<210> 24
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Fig. 22:
Mutagenic oligonucleotides for inactivating
carbohydrate binding sites in rMLB. - pT7 EcoRV to
SspI. -

<400> 24
cttcctttt caatattatt gaagcattta tcagg

35

<210> 25
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Fig. 22:
Mutagenic oligonucleotides for inactivating
carbohydrate binding sites in rMLB. - pT7 SspI to
EcoRV. -

<400> 25

cttccttttt cgatatcatt gaagcattta tcagg

35

<210> 26

<211> 40

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Fig. 23:
Mutagenic oligonucleotides for constructing
modular ITF gene cassettes. - pT7 Delta NdeI to
StuI. -

<400> 26

ctttaagaag gagatataca ggcctacgag aggctaagac

40

<210> 27

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Fig. 23:
Mutagenic oligonucleotides for constructing
modular ITF gene cassettes. - rMLB silent NheI. -

<400> 27

gttacctgca gtgctagcga acctacgggtg cgg

33

<210> 28

<211> 32

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Fig. 23:
Mutagenic oligonucleotides for constructing
modular ITF gene cassettes. - rMLA Delta AgeI. -

<400> 28

cccaccagac caccggcgaa gaatatttcc gg

32

<210> 29

<211> 40

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Fig. 23:
Mutagenic oligonucleotides for constructing
modular ITF gene cassettes.

<400> 29

gtttgtatgc ggagagcgtc cctcgagctc tgagggtcgc

40

<210> 30

<211> 43

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Fig. 23:
Mutagenic oligonucleotides for constructing
modular ITF gene cassettes. - rMLB Delta EcoNI to
AgeI. -

<400> 30

ccgaataatc atcgtccgg ccaccggtac accaaatcaa atg

43

<210> 31

<211> 11

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Flanking region
of the ProML gene cassette in expression vector
pT7ProML

<400> 31

tacatatgta c

11

<210> 32

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Flanking region
of the ProML gene cassette in expression vector
pT7ProML

<400> 32

ccatgataag gatcctctag

20

<210> 33

<211> 9

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Flanking region
of the IML gene cassette in expression vector
PIML-02-P

<400> 33

caggcctac

9

<210> 34

<211> 34

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Flanking region
of the IML gene cassette in expression vector
PIML-02-P

<400> 34

cactagtaac tcctcggatc ctctagagtc gacc

34

<210> 35

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Modulator
module peptide

<400> 35

Lys Asp Glu Leu

1

<210> 36

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Modulator
module peptide

<400> 36

His Asp Glu Leu

1

<210> 37

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Portion of the
ML propeptide

<400> 37

Ser Ser Ser Glu Val Arg Tyr Trp Pro Leu Val Ile Arg Pro Val Ile

1

5

10

15

INS
DI
C8
Ala

<210> 38

<211> 13

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: A degradation product of myelin basic protein.

<400> 38

Val His Phe Phe Lys Asn Ile Val Thr Pro Arg Thr Pro
1 5 10